

Sortase A

Week 10

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1	Colony PCR GB1-SortA	2
2	Liquid cultures and Protein expression GS1, GS2 from glycerol stocks	3

1 Colony PCR GB1-SortA

Responsible

Aman Mebrahtu

Protocols used

PCR Amplification using Taq Polymerase

Modifications and comments to protocols

No modifications were made to the protocol. Samples used: GS1, GS2, from Plates. Primers used: GS_F , GS_R .

Experimental Set Up

Table 1: Experimental set-up for colony PCR on all samples.

Mix 1	Component	Volume [μ l]
	PCR Grade Nucleotide mix	1
	F_Primer	5
	R_primer	5
	Template DNA	Glycerol stock
	Water	14
Mix 2	PCR reaction buffer 10x	5
	Taq DNA Polymerase	1.25
	Water	18.75
Total Voume		50

Results and Conclusions

Results after gelelectrophoresis deemed the colony PCR unsuccessful. Bands at sizes equivalent to what was expected (700 bp), cannot be observed on the gel. Unspecific bands are observed around 500 bp,

Discussion and Troubleshooting

The unspecific bands might be due to contamination or unspecific binding of primers used in the PCR. Thus the results are inconclusive and no conclusion can be drawn regarding of the right fragment type has been ligated into the vector backbone.

References

2 Liquid cultures and Protein expression GS1, GS2 from glycerol stocks

Responsible

Aman Mebrahtu

Protocols used

Protein expression

Modifications to protocols

No modifications were made to the protocol. Liquid cultures were made in 10 ml media with GS samples from glycerol stock. Protein expression was induced with 1 mM IPTG in 10 ml LB media and 12.5 μ l chloramphenicol [25 μ g/ml].

Experimental Set Up

Table 2: Samples used for Liquid cultures and Protein expression.

Flask No.	Sample
1	GS1
2	GS2

Results and Conclusions

SDS-PAGE results after protein expression were not obtained due to damaged gels. Complementing sequencing results of samples were inconclusive thus these samples were discarded.

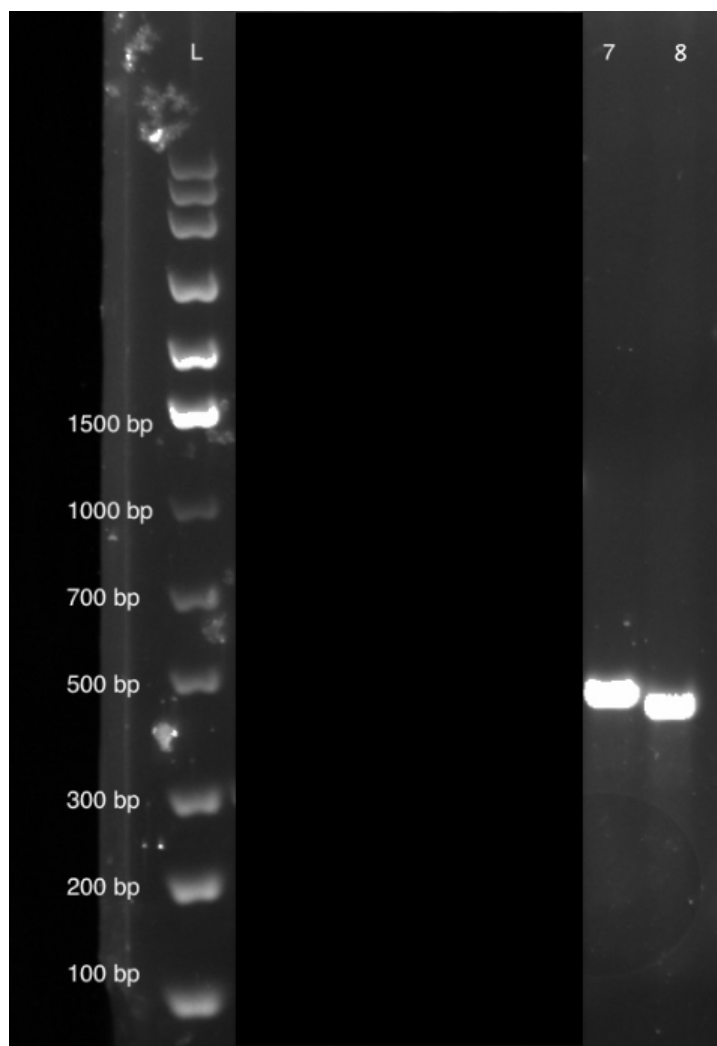


Figure 1: Result from gel electrophoresis in 1 % agarose, at 110 V. (L) Ladder Ladder, (7) GS1,(8) GS2.